53

Synergistic Control of Acute GvHD: Effectively Down-Regulating T Cell Proliferation and Cytotoxicity with Combined mTOR Inhibition and CD28:CD80/86 Costimulation Blockade

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Introduction: We have created a non-human primate (NHP) model of GvHD. Here we determined whether mTOR inhibition with sirolimus and CD28:CD80/86 costimulation blockade with belatacept could combine synergistically to prevent this disease.

Methods: Rhesus macaques were irradiated (9.6 Gy), and then transplanted with GCSF-mobilized PBSC from a haploidentical donor. Recipients were treated with either sirolimus alone, belatacept alone or combination therapy. Clinical GvHD was monitored using an NHP grading scale and multiparameter flow cytometric analysis (MFC) was performed.

Results: Untreated controls (n = 5) developed rapid, severe aGvHD and succumbed rapidly (MST = 7 days). Treatment with either sirolimus or belatacept alone partially protected against GvHD. Sirolimus-treated recipients (n = 6) developed predominantly GI disease and had an MST of 14 days (Figure 1A). Recipients treated with belatacept alone (n = 3) developed primarily liver aGvHD and had an MST of 11 days. In striking contrast, recipients treated with combined sirolimus + belatacept (n = 5) demonstrated neither uncontrolled diarrhea nor hyperbilirubinemia at the timed terminal analysis (1 month post-transplant).

We used MFC to measure the immunologic consequences of sirolimus and belatacept on T cell proliferation (Ki-67) and cytotoxity (granzyme B). While untreated aGvHD was associated with rampant CD8+ proliferation (with 83% Ki-67+ CD8+ T cells vs 4.7% pre-transplant), sirolimus or belatacept monotherapy partially controlled proliferation (35% and 65% Ki-67+ with sirolimus or belatacept respectively). Combined sirolimus + belatacept dramatically reduced proliferation (to 8%, favorably comparing with 13% Ki-67+ using tacrolimus/MTX).

Sirolimus and belatacept also partially controlled T cell cytotoxicity: While untreated aGvHD was associated with excessive CD8+ granzyme B expression (82% granzyme $B^{very\ high}$ vs 0.3% pre-transplant) sirolimus or belatacept monotherapy partially controlled cytotoxicity (8% and 35 % granzyme $B^{very\ high}$ with sirolimus or belatacept respectively). Combination therapy dramatically reduced granzyme $B^{very\ high}$ expression, to 1.5%, favorably comparing with 4% using CNI/MTX.

The ability of sirolimus, belatacept, or the combination to control Ki-67 and Granzyme B expression closely correlated with survival (Figure 1B,C), and significant co-expression of granzyme B in the Ki-67+ cells was observed (Figure 1D), suggesting that dual-positive Ki-67/Granzyme B cells may mark a pathogenic population, amenable to tracking in the peripheral blood.

Implications: These results show, for the first time, that sirolimus and belatacept can combine synergistically to control primate aGvHD. They also identify CD8+/Ki-67+/Granzyme $B^{very\ high}$ dual-positive T cells as a potentially sensitive biomarker of GvHD pathogenesis, amenable to monitoring longitudinally in the blood.

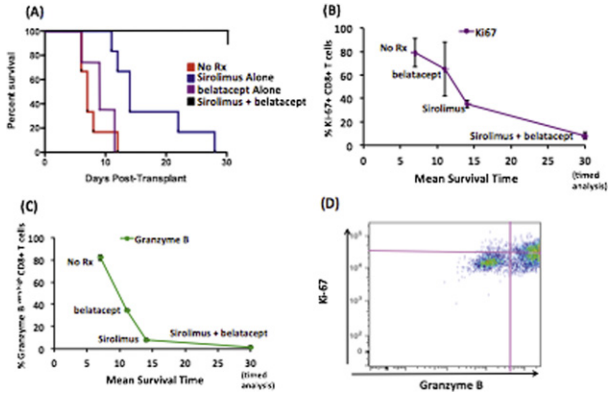

Figure 1. Synergistic Control of Acute GvHD With Sirolimus + belatacept
(A) Dual therapy with sirolimus + belatacept synergistically prolongs survival in a NHP aGvHD model.
(B) Proliferation, measured by Ki-67+ CD8+ T cells, correlates with clinical aGvHD severity.
(C) Cytoxicity, measured by Granzyme B expression in CD8+ T cells, correlates with clinical aGvHD severity.
(D) Significant co-expression of KI-67 and Granzyme B in pathogenic CD8+ T cells.

54

Evidence for Expansion of $CD21^{-}$ B Cells with an Exhausted Phenotype in Patients with Active Chronic GvHD

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Chronic graft versus host disease (cGvHD) remains a major complication of allogeneic hematopoietic stem cell transplantation (HSCT). At present, the immunopathophysiology of cGVHD is not fully understood. A number of preclinical and clinical studies support a role for B cell involvement in the pathogenesis of cGvHD. Increased numbers of $CD21^{-}$ B cell, believed to be immature/ transitional B cells, were recently reported in the peripheral blood (PB) of cGvHD patients. In this study, we report the expansion of a $CD21^{-}$ B cell population with an exhausted phenotype in the memory B cell compartment of patients with active cGVHD. Using multicolor flow cytometry we performed an extensive anaylsis of B phenotype and function in 16 patients with active cGvHD compared to 14 age-matched HSCT recipients without clinical evidence of cGVHD and 11 healthy controls. Chronic GvHD patients had significantly higher frequencies of $CD21^{-}$ B cells in the PB compared with patients without cGvHD and HC (median 12.2% vs. 2.12% vs. 3%; $P < 0.01$). Multi-parameter flow cytometry revealed that the majority of these cells were $CD10^{-}$ $CD27^{-}$ $CD21^{-}$ $CD20^{hi}$, reminiscent of the recently described exhausted B cells in HIV patients with chronic viremia. We did not observe increased transitional ($CD24^{hi}$-$CD38^{hi}$- $CD19^{+}$) or immature B cells ($CD10^{+}CD21^{-}CD27^{-}CD19^{+}$) in cGvHD patients compared with patients with no cGVHD (median transitional 3.4% vs. 6.3%; p=*0.08*) and (median immature (out of total $CD21^{-}$ B cells)